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Insights into the molecular interaction between sucrose and α-chymotrypsin

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ABSTRACT

One of the most important purposes of enzyme engineering is to increase the thermal and kinetic stability of enzymes, which is an important factor for using enzymes in industry. The purpose of the present study is to achieve a higher thermal stability of α-chymotrypsin (α-Chy) by modification of the solvent environment. The influence of sucrose was investigated using thermal denaturation analysis, fluorescence spectroscopy, circular dichroism, molecular docking and molecular dynamics (MD) simulations. The results point to the effect of sucrose in enhancing the α-Chy stability. Fluorescence spectroscopy revealed one binding site that is dominated by static quenching. Molecular docking and MD simulation results indicate that hydrogen bonding and van der Waals forces play a major role in stabilizing the complex. T_m of this complex was enhanced due to the higher H-bond formation and the lower surface hydrophobicity after sucrose modification. The results show the ability of sucrose in protecting the native structural conformation of α-Chy. Sucrose was preferentially excluded from the surface of α-Chy which is explained by the higher tendency of water toward favorable interactions with the functional groups of α-Chy than with sucrose.

1. Introduction

Enzymes are biological catalysts that can enhance the rate of biochemical reactions by several orders of magnitude with high specificity, making them essential tools in biomedical, chemical and industrial applications. The crucial importance of enzymes in the industry and medical applications stems from their stability and functional dimensions, making their study a subject of active research. During the past decades, utilization of enzymes with high thermal stability has attracted a large interest in academic and industrial applications [1–3]. Therefore, there is an essential need to engineer enzymes that can function in unnatural environments such as low temperatures and pH, so that their full potential can be realized.

The main restriction for enzyme function in hard conditions is the stability of enzymes. Increasing enzyme stability is, therefore, a significant goal of protein engineering [4] and a determinant factor in commercial applications. Enzymes lose part of their activities when they are subjected to heat, extreme pH or proteases. During the last decades, investigation has focused on the improvement of enzymes action in the conditions in which they were to be used, and especially on the increment of their thermal and kinetic stability [5–7]. The production of heat-resistant enzymes would allow carrying out enzymatic reactions to higher temperatures, and therefore, increasing conversion rates and substrates solubility and reducing the risk of microbial growth and the viscosity of the reaction medium [8–10].

The modification of the solvent environment is One of the best approaches for incrementing the stability and activity of biocatalysts. Different methods exist for raising the enzyme stability. One of the best of this methods is the addition of some material such as inorganic salts, polyols, and sugars, for example, trehalose directly to the protein solution [9,11]. The structure and activity of enzyme against denaturation condition can be protecting efficiently by some of the polyols.

S1 family enzyme such as α-chymotrypsin (α-Chy) has attracted a great deal of attention to biomedical and bioengineering fields because of its catalytic activity under aqueous conditions [12]. α-Chy is a model protein that allows the study of the effect of adding co-solvents, such as carbohydrate or polyols, on the folding and unfolding processes. Thus, α-Chy was selected for this study. Different spectroscopic methods, and molecular dynamics simulations and docking used for evaluating of the interaction mechanism between sucrose and α-Chy. The quenching mechanism, the thermodynamic parameters,T_m, the

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